INVESTIGATING CAUSES OF *PHRAGMITES AUSTRALIS* COLONIZATION IN GREAT MARSH, PARKER RIVER NATIONAL WILDLIFE REFUGE

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EXECUTIVE SUMMARY

An exotic invasive plant, common reed (*Phragmites australis*), threatens the integrity of the Great Marsh by spreading rapidly through the marsh, displacing native plants, and impacting ecosystem services provided by native salt marsh habitat. In cooperation with USFWS and the Great Marsh Partnership, a study was conducted to determine key factors that may contribute to the success of this undesirable plant within one of the most ecologically significant salt marshes in New England. Our data suggest that salinity seems to be the strongest and most scientifically significant factor favoring *Phragmites* expansion in the marsh, suggesting that the entire northern portion of the marsh may be vulnerable to further *Phragmites* spread across native communities if no management actions are taken. Fortunately, the management actions currently in place (*i.e.* selective herbicide spraying) appear to be working as a short-term approach for control that is helping scientists and managers 'buy time' until a more comprehensive management approach can be developed. In fact, sprayed areas are re-vegetating with a diverse, native population of salt marsh plants with little to no re-growth of *Phragmites* in the majority of sites examined. We have identified a great deal of variability across the marsh that suggests there is a mosaic of salinity, micro-elevation and surficial hydrologic conditions that plays a role in the invasion. We believe this mosaic may offer opportunities for 'precision management' by identifying areas to prioritize for active management, thus affording more efficient use of resources, time and effort on the ground. The next logical steps should be continuation of existing management approaches in tandem with launching two additional studies: 1) detailed salinity and elevation mapping and 2) a hydrologic model that examines the influence of large-scale infrastructure salinity and flooding. Integrating fine-scale mapping of salinity, elevation and hydrology would form the foundation to develop large-scale management concepts. Such an approach would be a powerful tool to validate theoretical model output with practical on-the-ground science to identify, map and quantify the areas most susceptible to colonization and further spread by *Phragmites*.

INTRODUCTION

The Great Marsh is one of the most ecologically significant salt marsh systems in New England. Portions of this complex system are designated as a United States Fish and Wildlife Service (USFWS) National Wildlife Refuge, Long Term Ecological Research (LTER) site of the National Science Foundation, Massachusetts Area of Critical Environmental Concern (ACEC), and Important Bird Area (IBA) designated by the National Audubon Society for numerous species, including the salt marsh sparrow. Comprising over 20,000 acres of marsh, barrier beach, tidal river, estuary, mudflat, and upland islands it is the largest contiguous salt marsh north of Long Island, extending across the Massachusetts North Shore, from Gloucester to Salisbury (Figure 1). The Great Marsh is among the best studied salt marsh systems in our area as part of the Parker River National Wildlife Refuge (the Refuge) and LTER networks, yet the ever increasing spread of exotic, invasive common reed (*Phragmites australis*) which threatens the natural salt marsh community remains largely unexplained.

Traditional models of *Phragmites* invasion suggest this aggressive plant colonizes high marsh in a relatively predictable pattern originating from upland edges. Yet at disturbed sites, such as those impacted by tidal restrictions or fill, more complex invasion patterns have been observed with invasion vectors originating at upland edges, creek banks (Bart and Hartman 2002), or even seemingly random locations in the marsh interior (Bart et al. 2006). Earlier research on spread of *Phragmites* in the relatively undisturbed Plum Island Estuary (PIE) supported these observations (Burdick et al. 2001), but contrasts strongly with new observations in these areas that show many small *Phragmites* colonies establishing in random locations throughout the marsh interior as mapped by Phippen and Walker in 2008 and 2009. Recent observations indicate that *Phragmites* is now present throughout the marsh, both as large monotypic patches as well as low-density occurrences mixed with native species (Moore et al. 2010, Burdick et al. 2011).

Recognizing the increasing threat, the Refuge, together with the Great Marsh Partnership, has engaged in an active management plan to control *Phragmites*, employing an integrated approach of herbicide use and prescribed burning since 2007. This has minimized the invasion, but it has treated only the 'symptoms', not the root cause. Our team, representing a collaboration of scientists and managers, has been working together with the Refuge over the last several years to identify the factors that most significantly contribute to the invasion and have identified areas for further study. The recent, prolific spread of *Phragmites* in the Plum Island Estuary portion of Great Marsh, coupled with the lack of any obvious cause (*i.e.*, acute disturbance), warrants serious concern for the stability of native salt marsh at this site, with management implications that may affect critical ecosystem services and wildlife habitat functions and values (Roman 1978; Benoit and Askins 1999, Keller 2000, Rooth et al. 2003; Windham and Meyerson 2003, Chmura et al. 2012).

Building from the results of our prior, small-scale studies, we sought to conduct a focused, larger-scale investigation of the factors that control or contribute to the unusual and unpredictable pattern of *Phragmites* invasion in portions of Great Marsh. We combined several approaches to determine factors that most significantly contribute to the presence of *Phragmites* in Great Marsh, including pore water chemistry, marsh sediment composition and structure, and plant community diversity, distribution and change over time (including a specific focus on presence/absence of *Phragmites* above ground and belowground biomass).

The results of this research are intended to guide ongoing conservation and management activities at the site and include parameters useful in the further development and refinement of existing ecological simulation models for future site assessment, restoration and management recommendations for maintaining native salt marsh habitat and associated ecological functions.

METHODS

Site Selection -Sampling stations were expanded from our previous work to include marsh sample sites bound on the north by the Plum Island Turnpike and to the south by Pine Island Creek. Previous sampling stations monitored in 2009 and 2010 were limited to more northern and eastern sections of the marsh. The present effort increased the sampling stations in the Study Area to a total of twenty-six plots, including many stations established in prior years, resulting in greater spatial coverage of the marsh and thus, a more representative view of overall site conditions. In addition, ten new plots were established south of Pine Island Creek within a non-invaded Reference Area of the marsh to determine whether the northern Study Area has distinct environmental conditions favoring *Phragmites* invasion (Figure 1). New plots were established using a random approach, but where a new randomly-generated location was close, it was replaced by the position of the prior plot. Alignment with prior study plots was particularly valuable because it allowed for additional analysis of a subset of our data over a longer, three-year observation period. All plots were marked in the field with PVC flags or well arrays and each plot position was recorded using GPS.

Pore Water Salinity – Pore water salinity influences the composition of plants and plant communities in the marsh. Our 2009 and 2010 field data have illustrated significant seasonal fluctuations in pore water salinity concentrations (Moore et al. 2010, Burdick et al. 2011), but our sampling plots covered only a small portion of the total marsh area. We expected that this trend would extend across the site, and may be more pronounced in areas with particular topographic features or soil conditions that may contribute to differences we observed. Thus, as detailed above, we expanding on this earlier work and collected pore water at 36 plots throughout the Study and Reference marshes at three depth ranges (0-20, 40-60, 80-100cm) using permanent PVC well arrays. Well sets were sampled four times during the

growing season by project partners Peter Phippen and Geoff Walker, capturing spring-neap tidal cycles.

Surface Water Salinity – Surface waters strongly influence pore water salinity. Despite regular tidal flooding at the site, the influence of seasonal storm events and contributions from the Merrimack River can result in significant freshening of surface floodwaters, potentially affecting pore water chemistry. In the summer of 2010, our team tracked surface water salinity at select locations in the marsh using *in situ* data-logging devices (Aqua-Troll 100). These data were used to interpret and understand patterns of pore water salinity at the site.

Pore Water Chemistry – Pore water chemistry is known to affect plant zonation patterns in salt marshes particularly when combined with effects of flooding (Chambers et al. 1998, 2003; Konisky and Burdick 2004). Measures of sulfide concentration and redox potential provide evidence of pore water conditions within the root zone that can result in physiological stress to plants. Native salt marsh plants have been shown to be more tolerant of these conditions than *Phragmites* (Chambers et al. 1999; Seliskar et al. 2004). Accordingly, field data collection included pore water sampling of salinity, redox potential, pH and sulfides using the sipper method (Portnoy and Valiela 2004) twice in late June and late August, 2011 growing season at each station. Pore water sampling included two depths; shallow (5-20 cm) and intermediate (45-60 cm) collected using 1 mm I.D. stainless steel tubing paired with a 60cc syringe. Salinity, redox potential and pH were determined in the field, whereas water collected for sulfide concentration was fixed in the field and later determined colorimetrically using Cline's reagent (Cline 1969) at Jackson Estuarine Laboratory (JEL).

Soil Composition and Structure – Differences in soil composition and structure can affect surface flooding and groundwater influence on marsh pore water chemistry. Our initial visual inspection of soil cores in 2009 (Moore et al. 2010) did not reveal an obvious difference in peat composition, depth, or soil texture between native and *Phragmites*-invaded portions of the marsh. However, only a limited number of paired qualitative observations were conducted, representing a relatively small portion of the marsh. In 2011, we conducted an assessment of soil composition and structure of paired cores at 32 stations throughout the marsh using quantitative techniques to determine soil texture, and percent organic matter to a depth of 1 meter, corresponding with maximum root depth for *Phragmites* (Moore et al. *in review*). Cores were collected in early spring and processed throughout the remaining project period. These data were intended to provide insight on water movement and drainage within soils and add context for observed patterns in pore water chemistry along gradients in the marsh (i.e., distance from creeks, marsh surface elevation, etc.).

Assessment of Vegetation Management - Our 2009 and 2010 data indicate significant differences in pH, Eh and sulfides between treated and untreated stands of *Phragmites* (Moore et al. 2010, Burdick et al. 2011). These data suggest treated sites

develop conditions less suited for re-colonization than in untreated sites. We examined edaphic conditions via pore water sampling (methods above) and re-growth of *Phragmites* using visual estimates of species diversity and percent cover in treatment areas as described below.

Vegetation Monitoring - Permanent vegetation quadrats were established along transects running from each pore water monitoring plot to the nearest adjacent water course (*i.e.,* creek or ditch). The transect line was positioned 2 meters from the well station to avoid trampling. Ditches were a minimum of 0.8 m wide and 0.5 m deep to be considered water courses. Vegetation was sampled in plots both 2 m from the center of each station and 1 m from the nearest water course in early summer and again in late summer of 2011. Visual estimates of percent cover were made for each species detected within 1 m^2 quadrats. Cover of wrack, dead material from previous years and bare areas were summed with the live plant cover to equal 100%. Plot canopy height was estimated as the height exceeded by 20% of the shoots. Stem density of *Phragmites* was counted and heights of the 3 tallest shoots were measured to the nearest centimeter, noting the presence or absence of flowers in late summer. In addition to plot sampling, radial vegetation transects in seven *Phragmites* stands that were established in 2010 were re-surveyed using a similar protocol. Here 1 m^2 quadrats were placed along a radial transect in three locations: at the stand center, the inner edge (*Phragmites* > 95% of plant cover), and the outer edge (*Phragmites* < 5% of plant cover). In addition, the distance to the outermost *Phragmites* shoot within 1 meter of the transect line was noted. In 2010 the quadrats were 0.5 m^2 in area and plant cover was not assessed.

Strategies for Accessing Resources – The ability of Phragmites to establish roots and rhizomes at depth may play a role in its success in salt marshes (Moore et al. *in review*). To test this hypothesis, we determined the presence and distribution of live roots and rhizomes of *Phragmites* in soil. A series of soil cores were collected to a depth of 1m within well-established monotypic stands of *Phragmites* to control for inclusion of live root biomass from other species. Core profiles were cut into 20 cm sections and a 5 cm subsample from each was rinsed through sieves to capture all root and rhizome material. Biomass was sorted into live and dead biomass. Resulting live material was dried and weighed to determine maximum root biomass, depth and overall distribution through the soil profile.

Data Analysis - Comparisons of spatial factors (e.g., geographic location, distance from creek), seasons, and *Phragmites* treatment type (control, herbicide) were made using separate one-factor ANOVA models (alpha = 0.05), with fixed effects on well salinity, pore water chemistry (salinity, redox potential, pH and sulfides), soils (bulk density, organic matter, root weight) and vegetation (richness and density). Unless otherwise specified, analyses involving pore water were made using data averaged over multiple sampling dates (annual means). Tukey's post hoc test was run to determine differences among means. Linear regressions were used to identify potential geographic spatial patterns in the dataset for spring and summer sampling efforts. Spatial regressions were run with data averaged over sampling depth or

date as specified. For all analyses, residuals were examined to ensure homogeneous variance and a normal distribution. Statistical significance for tests was set at an alpha level of 0.05 to control Type I error.

Data were further analyzed using several non-metric multivariate tests in PRIMER 6 version 6.1.9, including non-metric multi-dimensional scaling (MDS), hierarchical analysis (CLUSTER), analysis of similarity (ANOSIM), contributions to similarity analysis (SIMPER), and multivariate correlation involving plant community and pore water data (BEST). MDS and BEST analyses included plant community data at plots located 2 m away from station centers (well plots) in the late summer of 2011 in the form of percent cover data, and also pore water parameters (salinity, pH, redox potential, sulfides) that were collected at the same stations and during the same time period vegetation data were collected. Plant community data were standardized using a square-root transformation then analyzed as a Bray-Curtis similarity matrix. CLUSTER, ANOSIM and SIMPER used plant community data only. Pore water data were normalized then analyzed using Euclidean distances. For each comparison, MDS were run using 100 iterations and ANOSIM were run using 999 permutations.

RESULTS AND DISCUSSION

Pore Water Chemistry

Spatial and temporal differences were evident in soil salinity between Study and Reference Areas. These differences were seen in samples obtained from both sipper and well water collections. Overall, salinity obtained by a sipper was significantly higher in the southern Reference marsh at all depths than the Study Area (Table 1 and Figure 2a). The lower salinity in the Study Area initially suggests it is more suitable for *Phragmites* establishment than the Reference marsh to the south. When Study Area data were sorted by plots dominated by native plants vs. *Phragmites*, some important subtleties were revealed. It appears that the *Phragmites* plots account for most of the lower salinity values at all depths. Accordingly, the native plots in the Study Area, while somewhat lower in salinity, were not significantly different from the Reference sites (Figure 2b). These findings are particularly interesting because they demonstrate for the first time in three years of data collection that *Phragmites* populations are perhaps exploiting areas lower in salinity. Therefore, the next steps in our analysis were to examine how salinity varies over depth and time.

Well water salinity allowed for more detailed comparisons of Great Marsh because of higher frequency sampling and deeper water collections. Salinity collected from pvc wells are similar to the patterns from the sipper technique: Reference plots were more saline than the Study Area plots (Figure 3). Further examination showed that in fact, salinity increased throughout the summer months in the Reference Area (over all depths) (Figure 4a), but remained fairly steady within the Study Area for all but the shallow depth wells (Figure 4b). Deeper wells were most stable for salinity

overall, while shallow depth wells appear more influenced by surface flooding, which may be rather fresh, particularly in the spring and early summer.

When temporal comparisons of salinity were sorted by plant community in the Study Area, native stations again mirrored that of the Reference marsh, whereas salinity at *Phragmites* stations were stable over the summer months except at shallow depth (Figure 5a and b). Perhaps more important was that at deeper depths (intermediate and deep) pore water was always below 20 ppt within *Phragmites* stations, but greater than 20 ppt for 3 out of the 4 months within native stations. In areas dominated by *Phragmites*, not only is the overall salinity lower, but there is a constant source of fresher water throughout the growing season at deeper depths. Unlike native marsh plants, which roots are limited to the top 30 cm (Valiela and Teal 1974; Valiela et al. 1976; Gross et al. 1991; Steinke et al. 1996; McKee et al. 2006), *Phragmites* roots and rhizomes are able to penetrate the substrate up to and over 1 m (Haslam 1970, 1972; Lissner and Schierup 1997; Vretare et al. 2001; Ravit et al. 2006; Moore et al. *in review*). Therefore *Phragmites* can access this constant source of fresher pore water especially during the late summer months where surface and shallow salinity are higher. Our examination of soil cores in Great Marsh revealed live root and rhizomes to such depths, with live roots present up to 80 cm. Freshening of the marsh with spring rains helps *Phragmites* establish early in the season, often before native halophytes are biologically active, while the presence of fresher water at depths in excess of typical native species rooting depths (i.e., 50+ cm) later in the season may allow *Phragmites* to persist and thrive through fall. In contrast, areas of the study marsh typically *not* invaded by *Phragmites* maintain higher salinities at depth (>20 ppt), suggesting depth and seasonality of salinity are important factors influencing invasion.

So, in conclusion our expanded sampling strategy has delivered several important points. First, *Phragmites* has the ability to colonize throughout the Study Area, in contrast to the Reference Area. Secondly, variability in soil salinity over space and time in the Study Area appears to have limited establishment of successful *Phragmites* colonies to lower salinity areas, especially where salinity remains below 20 ppt throughout the growing season. A fine-scale mapping effort to define areas vulnerable to *Phragmites* invasion in the Study Area could help direct continued control (herbicide) efforts.

Overall, there appears to be little difference in pH between Reference and Study sites and no apparent effect of depth either. However, since pH inherently demonstrates low variation, the Study Area was significantly more acidic than the Reference site despite the small range (Table 1 and Figure 6a). Grouping the data by plant community type (native vs. *Phragmites*), we found that the native Study Area stations were influencing the differences observed: the pH of native plots was significantly lower than both *Phragmites* plots and Reference plots (Figure 6b). Lower pH values found for the native plots within the Study Area may be due to oxidation of accumulated sulfides associated with higher redox potentials (as

discussed below). Of the *Phragmites*-only plots, neither depth nor treatment status seemed to differ significantly (Figure 6c).

Redox potential and sulfides are inherently related measures, typically exhibiting an inverse correlation (Portnoy 1997, Chambers 1998). Accordingly, our analyses reflect this pattern and represent the expected condition, with soils accumulating greater sulfides in the Reference Area where redox potential was generally lower (Table 1, Figure 7a and 7b). The ranges of redox and sulfide shown are typical for a salt marsh, and the more oxidized conditions of the Study Area suggest a potential explanation or contributing factor to *Phragmites*' ability to do well at this site.

However, a closer look at the data grouped by plant community type (native plant vs. *Phragmites*-dominated plots) in the Study Area reveals that the variation between Reference and Study Areas must be attributed to the native plots (Figure 7c and 7d). In fact, redox potential and sulfides in *Phragmites*-invaded areas do *not* significantly differ from the Reference Area. In trying to understand and explain this unexpected trend, we noted that the native plots in the Study Area were disproportionately located at or near major creek edges (57% for native stations, 33% for *Phragmites* stations), a habitat which is known to be well drained and less reduced than mid-marsh locations. When redox potential and sulfide concentrations were analyzed after removing all plots within close proximity to major creeks, no differences were observed between native and *Phragmites* plots. While this observation helps explain the data, it doesn't provide us with insight into how *Phragmites* seems to advance within plots exhibiting sulfide levels that have been shown to give *Spartina* species the competitive advantage (0.9mM; Seliskar et al. 2004).

Despite the expanded dataset from previous years covering a larger geographic area, an analysis of pore water across the marsh revealed few strong spatial patterns for parameters other than salinity. No discernable relationships emerged between pore water and an east to west gradient (Figure 8). However, annual averages of pore water salinity collected by 'sipper' revealed a spatial trend, with an increase in salinity the further south sampled (r^2 = 0.17; Fig. 9). This relationship was stronger at the beginning of the summer at both shallow and mid depths (r^2: 0.38 and 0.26, respectively). The occurrence of *Phragmites* stands also follows this same general pattern. There were far more stands located in the Study Area versus the rarely invaded southern Reference marsh, limited to two diffuse stands in the marsh interior. Salinity is likely the dominant factor for the *Phragmites* distribution pattern we observed. Other parameters (pH, redox potential and sulfides) did not appear related to longitude (r^2: 0.01 to 0.06). Although no pattern other than salinity emerged when relating pore water to geography, the lack of relationship may be more important to understanding of invasion. An aerial view of invasion shows a high density of stands (> 100) north of Little Pine Island Creek without a distinct east to west pattern (Figure 1), similar to the findings of the numerous statistical regressions we performed to elucidate potential trends. It may be possible that the entire northern section of Great Marsh possesses conditions suitable for

Phragmites invasion and spread; therefore no distinct patterns in pore water were detected.

Pore water chemistry was further analyzed for spatial patterns relative to distance from tidal creeks and ditches. At shallow sampling depths, soil anoxia (redox potential and sulfides) was significantly related to distance from creeks (Figure 10). The relationship between soil anoxia and distance from creek was strengthened when the stations associated with ditches or minor creeks were excluded, since these smaller water features appear to have little impacts on pore water chemistry. The relationship with the remaining stations measured to major creeks (n=20) can be best characterized by a sharp decrease in redox potential (or increase in sulfides) within 20 m of a creek, followed by a more gradual decrease beyond 20 m. At deeper sampling depths (45-60 cm), the same pore water pattern was followed, with increased soil anoxia farther from creeks (Figure 11). Pore water salinity and pH were not significantly related to distance from creek regardless of water structure (creek or ditches). But note that stations associated with lesser water features never averaged over 20 ppt for shallow or mid depth pore water (Figures 10 and 11).

Similar to the findings of previous years, marsh areas distant from tidally influenced creeks (<10 m) contained over twice the amount of sulfides accumulating than areas relatively close to creeks at the shallow depth and about 70% more at mid depth (Table 2). Our results demonstrate once again that areas adjacent to major creeks are more suitable to invasion by *Phragmites*. These areas not only contain more desirable pore water conditions, but are also more often disturbed by wrack, a form of natural disturbance that often smothers and kills native salt marsh vegetation (Peter & Burdick 2010) and favors *Phragmites* establishment (Minchinton 2002).

Differences in pore water between stands of *Phragmites* treated with herbicides and those that were not did not appear to persist in the 3rd year of monitoring. In previous years, we found pore water in stands treated with herbicide to be less saline and more anoxic especially so in the 1st year post treatment, 2009 (Figure 12; Burdick et al. 2011). In 2011, there were no significant differences in any pore water parameters when depth is averaged (Figure 12), and only salinity at intermediate soil depths is significant when parsing out parameters by depth (Table 3). It appears much of the effect of herbicides on pore water chemistry is short term. The strongest differences were noted in 2009, but weakened the following year. In 2011, native plant communities have re-colonized significant portions of the treated area, perhaps counteracting the effect found in prior years. Nonetheless, it is evident that treating *Phragmites* in Great Marsh has benefits, but that this management approach is not a long-term solution.

<u>Vegetation</u>

One of the most interesting observations from our summary data was the remarkable difference in plant communities between Reference and Study Area. Table 4 shows the total diversity of the Reference Area with only five species, all of

which are common and familiar salt marsh macrophytes (column 1). In contrast, the Study Area boasts 23 species overall (column 2), which includes the same 5 from the Reference. The majority of additional species found in the Study marsh typically inhabit brackish environments (e.g., *Amaranthus cannabinus, Aster subulatas, Juncus balticus, Schoenoplectus maritmus*) and some are forb pannes specialists (e.g., *Glaux maritima*, *Plantago maritima, Triglochin maritima*; (Griffin et al. 2011). Of course, with more stations in the Study Area (n=26) versus the Reference marsh (n=10), we would expect more species in the Study Area because sampling area is directly related to species and habitat diversity, but this argument fails once the samples are subdivided into treated and untreated *Phragmites* plots. There are actually less treated or untreated plots of Phragmites leading to four fold greater species richness (Table 4). Among the sampled areas in the Study marsh, *Phragmites* stands showed the highest diversity (column 4 and 5), with no noticeable difference between treated and untreated stands. In addition to the lower salinity of the *Phragmites* invaded areas, herbicide treatment may act as a secondary succession disturbance, potentially allowing different species the opportunity to establish in these marsh areas, at least temporarily.

When the data are examined per station (# of species per plot), considerable differences in richness were also noted with twice the species found in the Study Area (Figure 13a). This difference is consistent through closer analysis when richness data is sorted by dominant vegetation type and treatment status (Figure 13b-c). In fact, these analyses revealed that the greatest richness per plot were found in treated *Phragmites* stands; evidence that management has a positive result beyond merely reducing *Phragmites* stem density and canopy. Figure 14 better represents the composition of treated and untreated plots, revealing a dramatic difference between the percent of *Phragmites* (treated = 15%, untreated = 77%) and native halophytes (treated = 73%, untreated = 3%) in particular. Treated plots also had higher cover of bare ground, but lower cover of wrack compared to untreated plots. The bare ground of treated plots may allow new species to establish by seed, leading to greater plant species richness. On untreated plots, the high wrack cover, which tends to kill native plants, seems to catch on the tall *Phragmites* stems, further reducing native species (Minchinton 2002, Burdick *pers obs*).

Differences between the Reference and Study Area plant communities were further examined using multivariate analyses. Plant cover data collected at station plots during late summer of 2011 reveal distinct communities using a non-metric multidimensional analysis (MDS; Figure 15). Reference stations are tightly clustered and distinctly different from Study Area stations. According to the results of an analysis of similarity (ANOSIM), the Reference Area is significantly different from each of the other three communities found in the Study Area, with the largest differences in plant community occurring with *Phragmites* areas (Treated R Statistic = 0.868, Untreated R Statistic = 0.831; Figure 16). In contrast, no significant differences were found between the three communities within the Study Area.

We also examined which species contributed most to community differences using SIMPER analysis. The species contributing most to community dissimilarity was *Spartina patens* (Table 5). The Reference Area averaged 62 % *S. patens* cover per 1 m^2 plot, whereas all three Study Area communities averaged only 2-4 %. Similarly, *Distichlis* cover was greater in Reference plots than in the Study Area, especially in *Phragmites* invaded areas. In the absence of *S. patens* and *D. spicata*, native areas had greater cover of a high marsh perennial (*Juncus gerardii*) and brackish forbs (represented by *Glaux maritima*) than found in the Reference Area (Table 5). In fact, *J. gerardii* was found in the greatest abundance in areas invaded by *Phragmites*, whether treated or not. Typically, *J. gerardii* tends to grow at upper elevations of the marsh, along creek levees and upper marsh areas, often in association with *Iva frutescens* (high tide bush). The co-occurrence of *J. gerardii* with *Phragmites* could mean that these species preferentially colonize the highest elevations of the Study Area, or that the elevation building ability of *Phragmites* (Windham and Lathrop 1999, Burdick and Konisky 2004) has increased marsh elevation to levels suitable for *J. gerardii*. Soil data support the latter explanation, as we found a trend towards higher organic matter accumulation in *Phragmites* stations (Figure 18).

Differences in vegetation communities between the Reference and Study Area did not translate when analyzing pore water parameters using MDS (Figure 17). No discernable patterns were evident among communities as indicated by the lack of significance (as indicated by ANOSIM). In addition, no strong relationships existed between pore water parameters and the vegetation community, with sulfide concentration at shallow depth being most correlated (0.129, as indicated by BEST analysis)

<u>Soils</u>

Paired soil cores were collected from each of the 32 plots, spanning Reference and Study Areas. Cores were characterized for soil organic matter, bulk density, and soil texture within 20 cm increments down the core to a maximum depth of 100 cm. In addition, a subset of these cores occurring in *Phragmites*-dominated sites were examined for the presence of live roots and rhizomes of *Phragmites*. Figure 18 summarizes the organic matter and bulk density results, which show no significant differences in these parameters between Study and Reference, nor for *Phragmites* presence or absence. However, as mentioned briefly above, the presence of *Phragmites* roots in soil cores to a depth of 80+ cm, suggests a potentially important characteristic of this invasive plant in Great Marsh. These data reflect the effective rooting depth of *Phragmites* in nearby Great Bay Estuary (Moore et al. *In Review*), which summarize data from 16 additional *Phragmites*-invaded salt marshes (Figure 19). When coupled with spatial and temporal trends in soil salinity, the ability of *Phagmites* to exploit greater soil depths than native halophytes may be more significant to its success in the marsh than other soil parameters we measured. While the depth of rooting cannot be controlled, it may be possible to affect processes that contribute to the salinity regime of this system, particularly if we gained a finer resolution understanding of pore water salinity patterns in key areas of the marsh (e.g., core areas of *Phragmites* invasion and associated

microtopographic relief that likely contributes to presence or absence of this species of concern.

Summary

One of our most significant results suggests the problem may actually be bigger than it looks. The invaded northern portion of Great Marsh is significantly fresher than the Reference Area of native marsh to the south. While other parameters measured show interesting trends, salinity seems to be the strongest and most scientifically significant factor favoring *Phragmites* expansion in the marsh, suggesting that the entire northern area may be vulnerable to further *Phragmites* spread across native communities if no management actions are taken. Areas within the northern marsh adjacent to major creeks appear even more vulnerable to invasion due to higher probability of disturbance and propagule dispersal, and greater soil oxidization resulting from drainage. Because *Phragmites* is an obligate out-crosser that is wind-pollinated, recent work along the mid-Atlantic seaboard (McCormack et al. 2010) suggests that once several genetically distinct populations co-occur, seed viability increases dramatically.

On a more positive note, our findings also suggest that on some level, herbicide-based management works, but it is evident that more management is needed. The management actions currently in place (*i.e.* spraying) appear to be working as a short-term approach for control that is helping scientists and managers 'buy time' until a more comprehensive management approach can be developed. In fact, sprayed areas are re-vegetating with a diverse, native population of salt marsh plants with little to no re-growth of *Phragmites* in the majority of sites examined. But this approach treats symptoms, not the underlying cause of habitat alteration that may favor *Phragmites* expansion overall.

Together, our findings suggest that further study needs to target salinity and elevation variability on a fine scale. Our work to date, which has been substantial, spans three years and a variety of factors affecting salt marsh functions and values. While it has not produced a management 'silver bullet' *per se*, it has pinpointed and confirmed the significance of salinity as the dominant factor between the invaded portions of Great Marsh and the adjacent natural Reference Area to the south. But the area is large, and we have identified a great deal of variability across the land surface that suggests there is a mosaic of salinity, micro-elevation and surficial hydrology that each plays a role in the patterns of invasion. We believe this mosaic may offer opportunities for 'precision management' by identifying areas of prioritization for active management, while affording more efficient use of resources, time and effort on the ground.

Thus, the next logical steps should be continuation of existing management approaches in tandem with launching of a detailed mapping and modeling initiative for the site. We can all agree we need to better understand the way water floods and mixes over the surface of Great Marsh, particularly since we have confirmed the

significance of salinity within the soils of this impacted system. Such a model, integrating hydrology and precision land survey would speak volumes about how salinity patterns are formed. These data would form the foundation for potential large-scale management concepts. Pairing this model with fine-scale mapping of salinity over key portions of the marsh using electromagnetic induction (a technology our team has recently developed) would be a powerful tool to validate the theoretical model output with practical on-the-ground science to *identify, map and quantify* the areas most susceptible to colonization and further spread by *Phragmites*.

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Table 1 - Comparison of pore water (salinity redox potential, sulfides, pH) grouped by location (study area vs. reference area) and sampling depth (shallow: 10-25 cm vs mid: 45-60 cm). Data was averaged over multiple dates to an annual average and sampled using sipper technique.

		Reference		Study		
		X	SE	X	SE	*p value*
Salinity (ppt)						
	Shallow	18.8	1.3	15.4	0.7	*0.01*
	Mid	21.3	1.1	18.0	0.8	*0.01*
Redox Potential (mV)						
	Shallow	-193	30	-147	19	*0.23*
	Mid	-247	24	-182	21	*0.09*
Sulfides (mM)						
	Shallow	1.5	0.3	1.0	0.2	*0.20*
	Mid	2.0	0.3	1.4	0.2	*0.10*
pH						
	Shallow	6.72	0.06	6.48	0.05	*0.01*
	Mid	6.68	0.04	6.33	0.09	*0.02*

Table 2 - Comparison of pore water (salinity redox potential, sulfides, pH) grouped by distance to creeks and sampling depth (shallow: 10-25 cm vs mid: 45-60 cm). Data was averaged over multiple dates to an annual average and sampled using sipper technique.

	Distance from Creek (m)									
Shallow Depth	Creek Edge	SE	4 to 10	SE	10 to 20	SE	20 +	SE	F ratio	p value
Salinity (ppt)	15.1	0.8	16.7	1.4	16.7	1.5	16.7	1.3	0.46	0.712
Redox Potential (mV)	-88.0	29.5	-100.7	33.4	-175.4	34.2	-262.9	17.9	8.25	<0.001
pH	6.53	0.07	6.45	0.10	6.55	0.10	6.60	0.07	0.64	0.593
Sulfides (mM)	0.55	0.23	0.71	0.24	1.39	0.34	1.93	0.25	6.09	0.001
Mid Depth										
Salinity (ppt)	16.6	1.1	19.9	1.3	19.8	1.5	19.2	1.6	1.69	0.178
Redox Potential (mV)	-140.4	29.0	-150.0	37.3	-202.4	36.7	-306.3	8.9	6.26	<0.001
pH	6.26	0.19	6.29	0.15	6.51	0.09	6.66	0.04	1.91	0.136
Sulfides (mM)	0.92	0.27	1.43	0.27	1.48	0.40	2.45	0.25	4.56	0.006
n	*9*		*9*		*8*		*11*			

Table 3 - Comparison of pore water (salinity redox potential, sulfides, pH) grouped by management of *Phragmites* (untreated and treated with herbicides) and sampling depth (shallow: 10-25 cm vs mid: 45-60 cm). Data was averaged over multiple dates to an annual average and sampled using sipper technique.

		Untreated		Treated		
		X	SE	X	SE	*p value*
Salinity (ppt)						
	Shallow	13.5	1.0	15.3	1.3	*0.33*
	Mid	14.0	0.9	19.9	1.6	*0.02*
Redox Potential (mV)						
	Shallow	-217	52	-126	46	*0.22*
	Mid	-245	70	-217	48	*0.74*
Sulfides (mM)						
	Shallow	1.7	0.5	0.8	0.1	*0.21*
	Mid	2.3	0.7	1.4	0.5	*0.29*
pH						
	Shallow	6.66	0.10	6.56	0.44	*0.52*
	Mid	6.60	0.16	6.58	0.10	*0.92*

Table 4 - Total floral diversity for Reference areas (column 1) vs. Study Areas (columns 2-5) separated by dominant plant community and treatment status.

Reference	Study			
	All Areas	**Native**	***Phragmites* Treated**	***Phragmites* Untreated**
Distichlis spicata	*Agalinas maritima*	*Argentina anserina*	*Agalinas maritima*	*Agalinas maritima*
Juncus gerardii	*Amaranthus cannabinus*	*Atriplex patula*	*Amaranthus cannabinus*	*Amaranthus cannabinus*
Salicornia depressa	*Argentina anserina*	*Distichlis spicata*	*Argentina anserina*	*Argentina anserina*
Spartina alterniflora	*Aster subulatas*	*Glaux maritima*	*Aster subulatas*	*Aster subulatas*
Spartina patens	*Aster tenufolius*	*Juncus gerardii*	*Aster tenufolius*	*Aster tenufolius*
	Atriplex patula	*Plantago maritima*	*Atriplex patula*	*Atriplex patula*
	Distichlis spicata	*Puccineliia maritima*	*Distichlis spicata*	*Distichlis spicata*
	Glaux maritima	*Schoenoplectus robustus*	*Glaux maritima*	*Glaux maritima*
	Iva frutescens	*Solidago sempervirens*	*Iva frutescens*	*Iva frutescens*
	Juncus balticus	*Spartina alterniflora*	*Juncus gerardii*	*Juncus balticus*
	Juncus gerardii	*Spartina patens*	*Phragmites australis*	*Juncus gerardii*
	Plantago maritima	*Triglochin maritimum*	*Plantago maritima*	*Phragmites australis*
	Phragmites australis		*Puccineliia maritima*	*Puccineliia maritima*
	Puccineliia maritima		*Salicornia depressa*	*Polygonum ramosissimum*
	Polygonum ramosissimum		*Schoenoplectus maritimus*	*Salicornia depressa*
	Salicornia depressa		*Schoenoplectus robustus*	*Schoenoplectus americanus*
	Schoenoplectus americanus		*Solidago sempervirens*	*Schoenoplectus maritimus*
	Schoenoplectus maritimus		*Spartina alterniflora*	*Solidago sempervirens*
	Schoenoplectus robustus		*Spartina patens*	*Spartina alterniflora*
	Solidago sempervirens		*Triglochin maritimum*	*Spartina patens*
	Spartina alterniflora			*Triglochin maritimum*
	Spartina patens			
	Triglochin maritimum			
Totals: 5	23	12	20	21

Note: Survey of well plots only

Table 5 – Results of a species of contributions to similarity analysis (SIMPER), showing the largest contributing species to dissimilarity among significantly different communities, as determined by an ANOSIM.

Species	**Average Cover (%)**		**Contribution to Dissimilarity %**	**Cumulative Contribution**
Reference vs. *Phragmites* Treated (Avg. dissimilarity 73.7 %)	Reference	PA Treated		
Spartina patens	61.94	2.34	20.62	20.62
Juncus gerardii	0.05	22.18	14.67	35.29
Distichlis spicata	17.89	8.64	9.78	45.07
Glaux maritima	0.00	8.35	9.30	54.37
Phragmites australis	0.00	4.20	6.62	60.99
Reference vs. *Phragmites* Untreated (Avg. dissimilarity 68.7 %)	Reference	PA Untreated		
Spartina patens	61.94	3.88	21.63	21.63
Juncus gerardii	0.05	26.42	17.68	39.31
Phragmites australis	0.00	7.13	9.72	49.02
Glaux maritima	0.00	7.62	9.64	58.66
Distichlis spicata	8.46	12.11	8.62	67.29
Reference vs. Study- Native (Avg. dissimilarity 65.2 %)	Reference	Study- Native		
Spartina patens	61.94	4.04	26.38	26.38
Distichlis spicata	17.89	17.14	16.08	42.45
Glaux maritima	0.00	8.76	12.5	54.95
Juncus gerardii	0.05	8.24	12.25	67.2

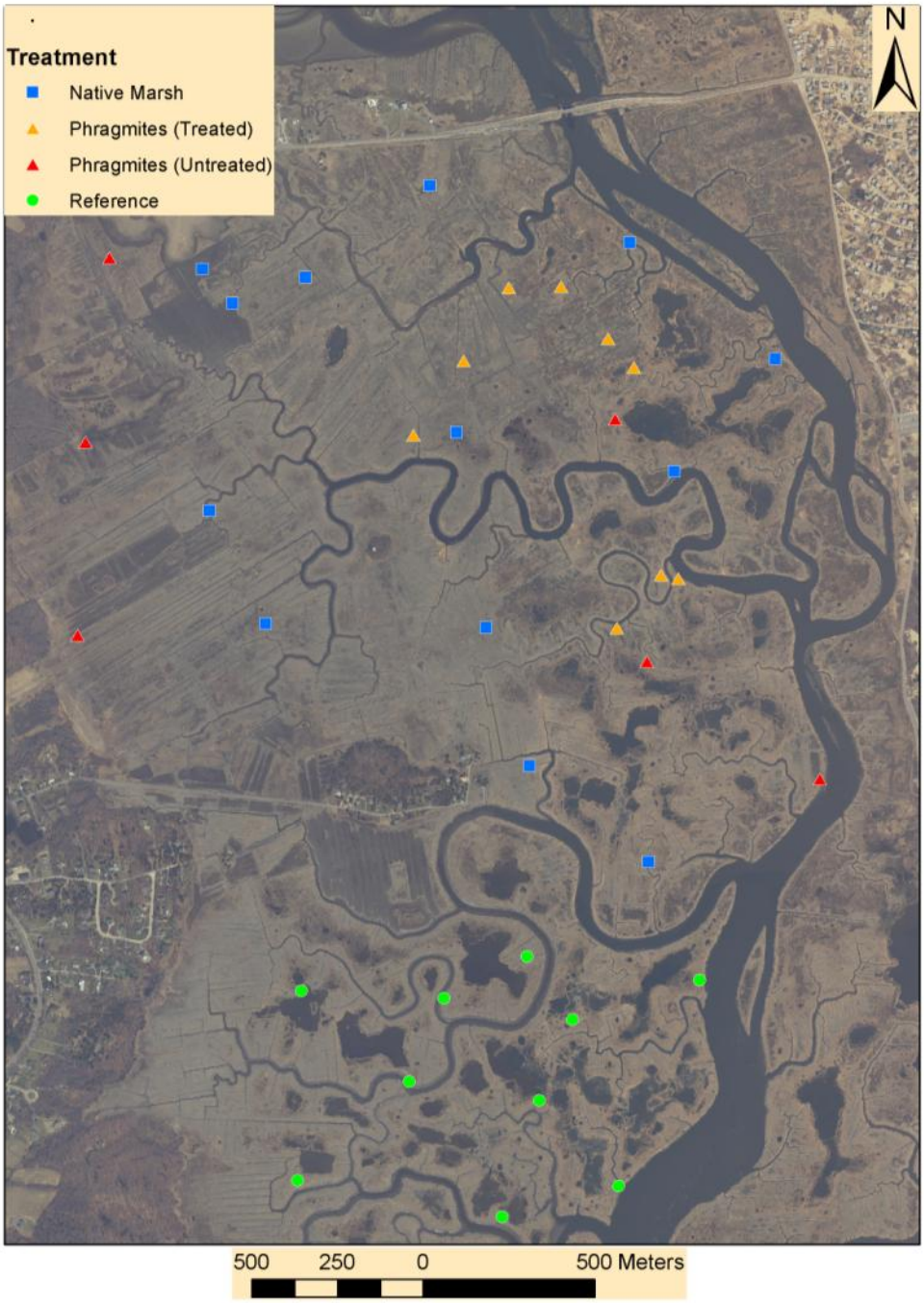

Figure 1 - Location of study and reference area random stations established and sampled in 2011. Green circles are stations in the reference area, whereas all other stations are in the study area.

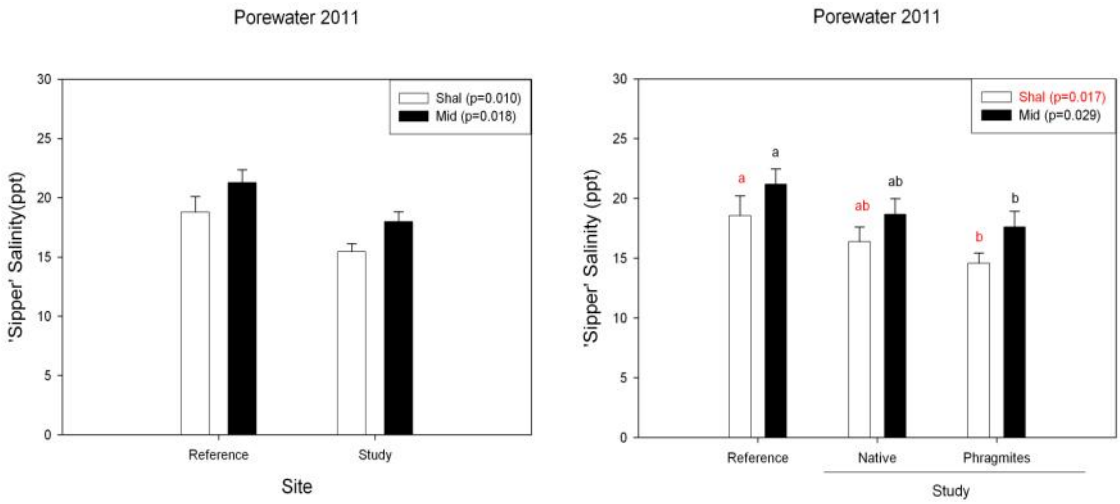

Figure 2 - Sipper salinity by (a) location and salinity by (b) location sub-grouping in the study area, native vs. *Phragmites* plots. Data was averaged over multiple dates to an annual average and analyzed over depth (shallow: 10-25 cm vs mid: 45-60 cm). As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

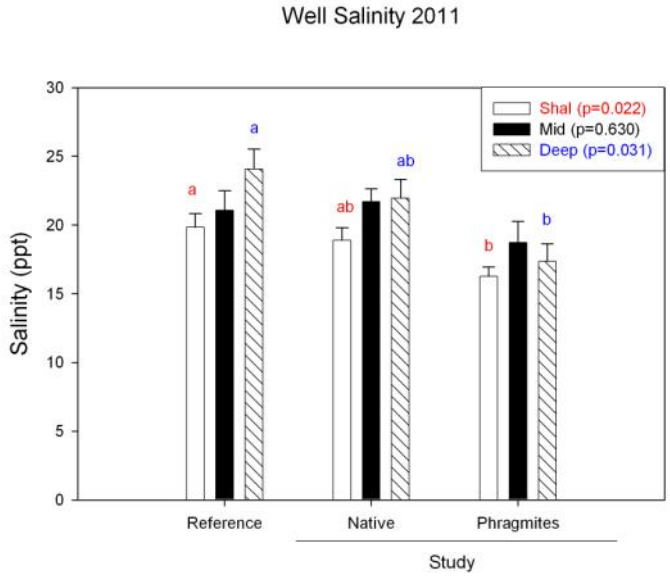

Figure 3 - Comparison of salinity collected from a pvc well between reference and study areas (native vs. *Phragmites* dominated community) across depth (shallow: 10-25 cm vs mid: 45-60 cm, deep: 85-100 cm). Data was averaged over multiple dates to an annual average. As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

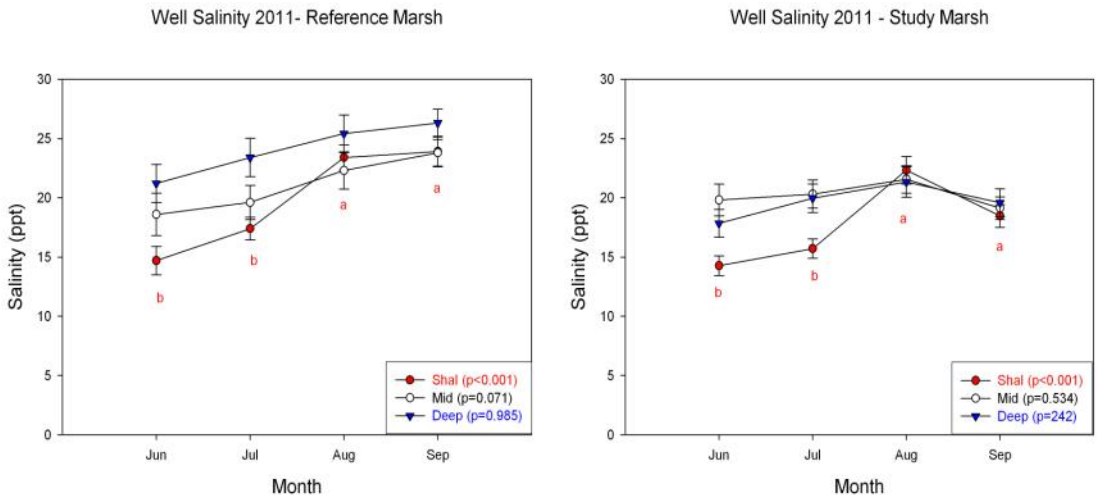

Figure 4 - Well salinity by month and depth (shallow: 10-25 cm vs mid: 45-60 cm, deep: 85-100 cm) for (a) reference and (b) study area. As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

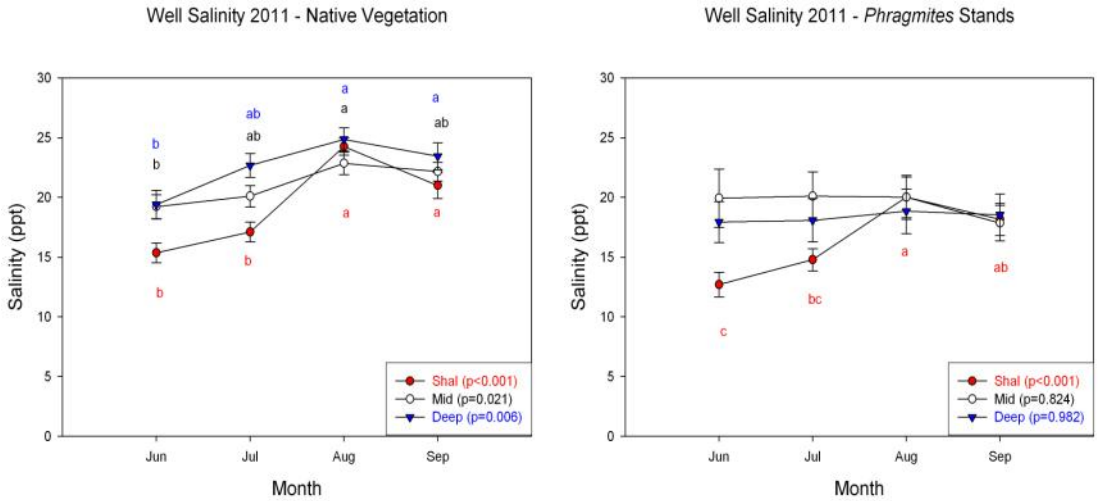

Figure 5 - Well salinity by month and depth (shallow: 10-25 cm vs mid: 45-60 cm, deep: 85-100 cm) for areas of the marsh dominated by (a) native species and by (b) *Phragmites* in the study area. As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

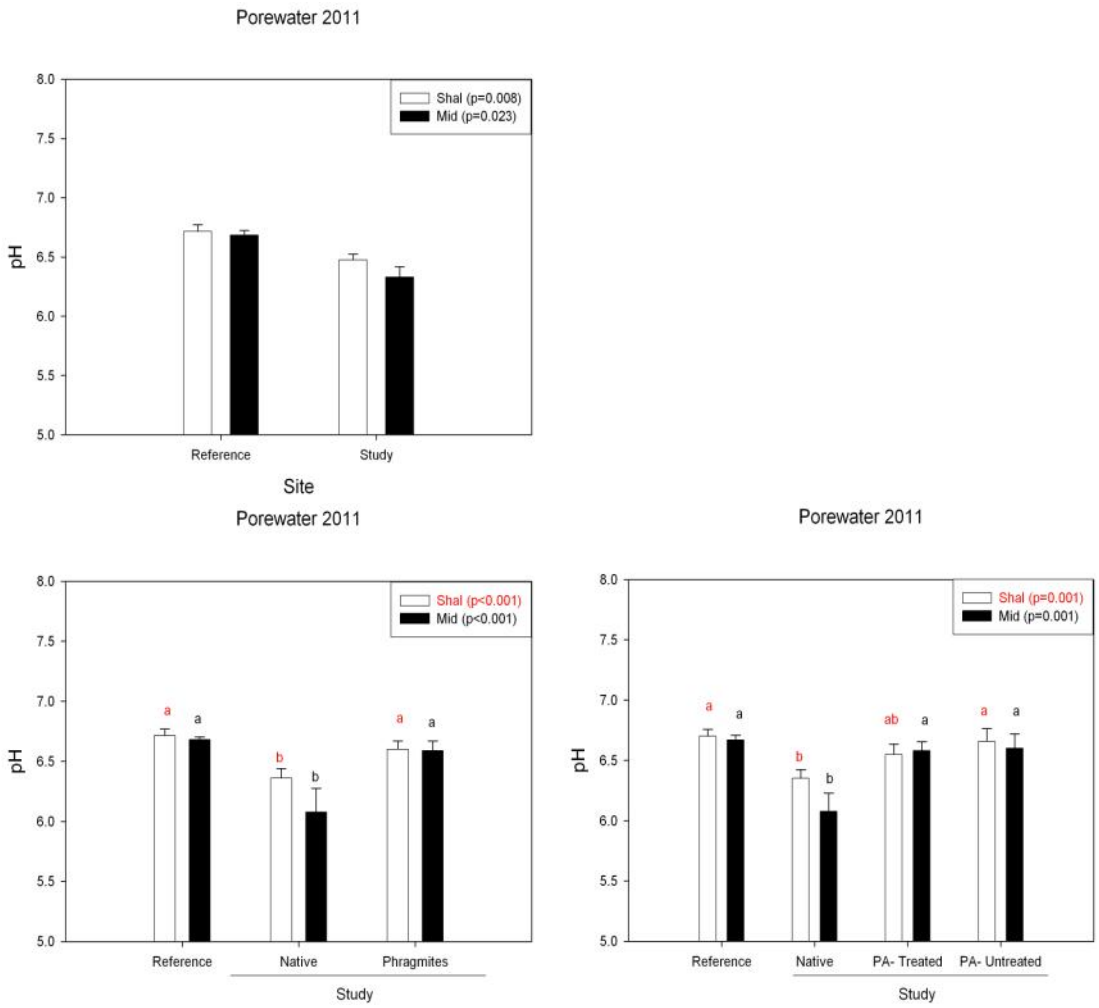

Figure 6 - Comparison of pH by (a) location and by (b-c) location sub-grouping in the study area, native vs. *Phragmites* plots (herbicide-treated, untreated). Data was averaged over multiple dates to an annual average and analyzed over depth (shallow: 10-25 cm vs mid: 45-60 cm). As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

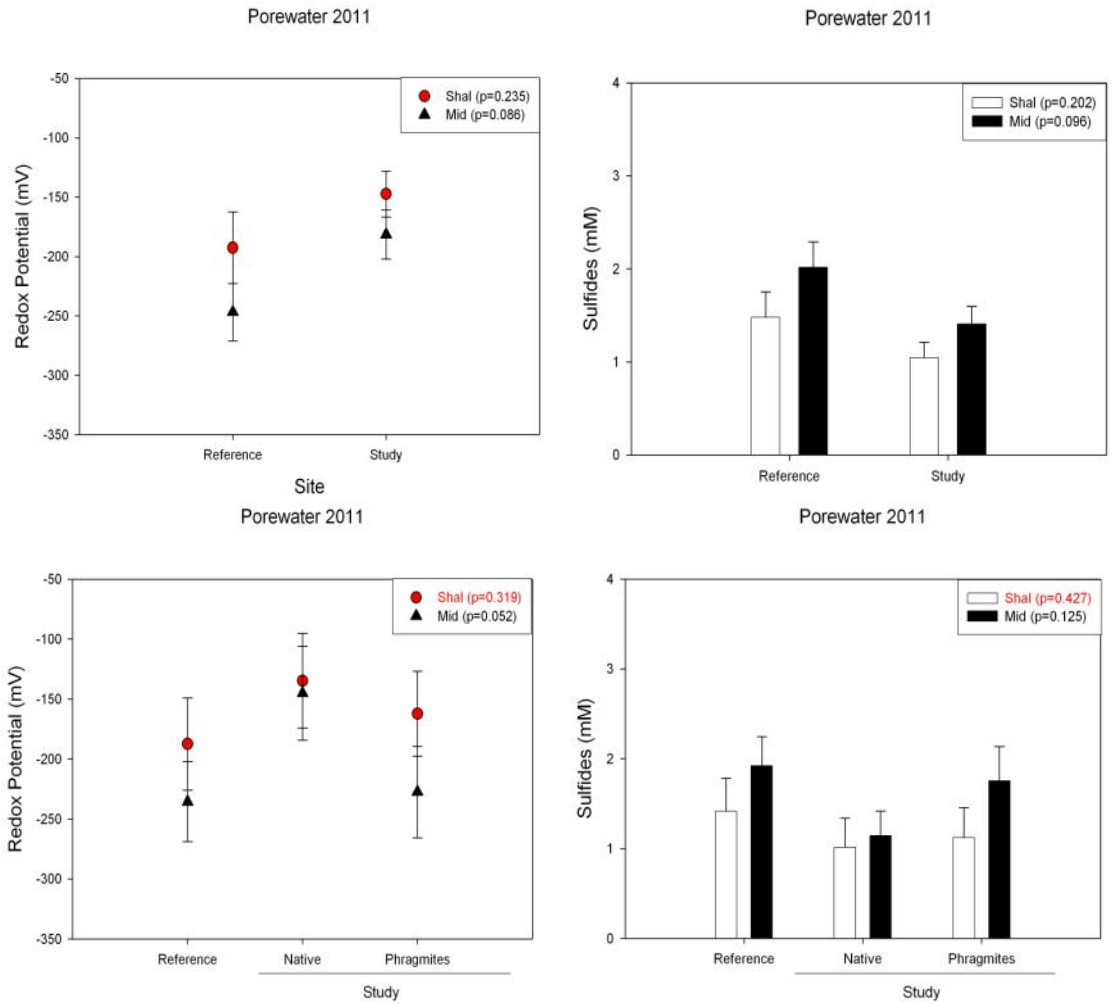

Figure 7 – Comparison of redox potential and sulfides by (a-b) location and by (c-d) location sub-grouping in the study area, native vs. *Phragmites* plots (herbicide-treated, untreated). Data was averaged over multiple dates to an annual average and analyzed over depth (shallow: 10-25 cm vs mid: 45-60 cm). As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

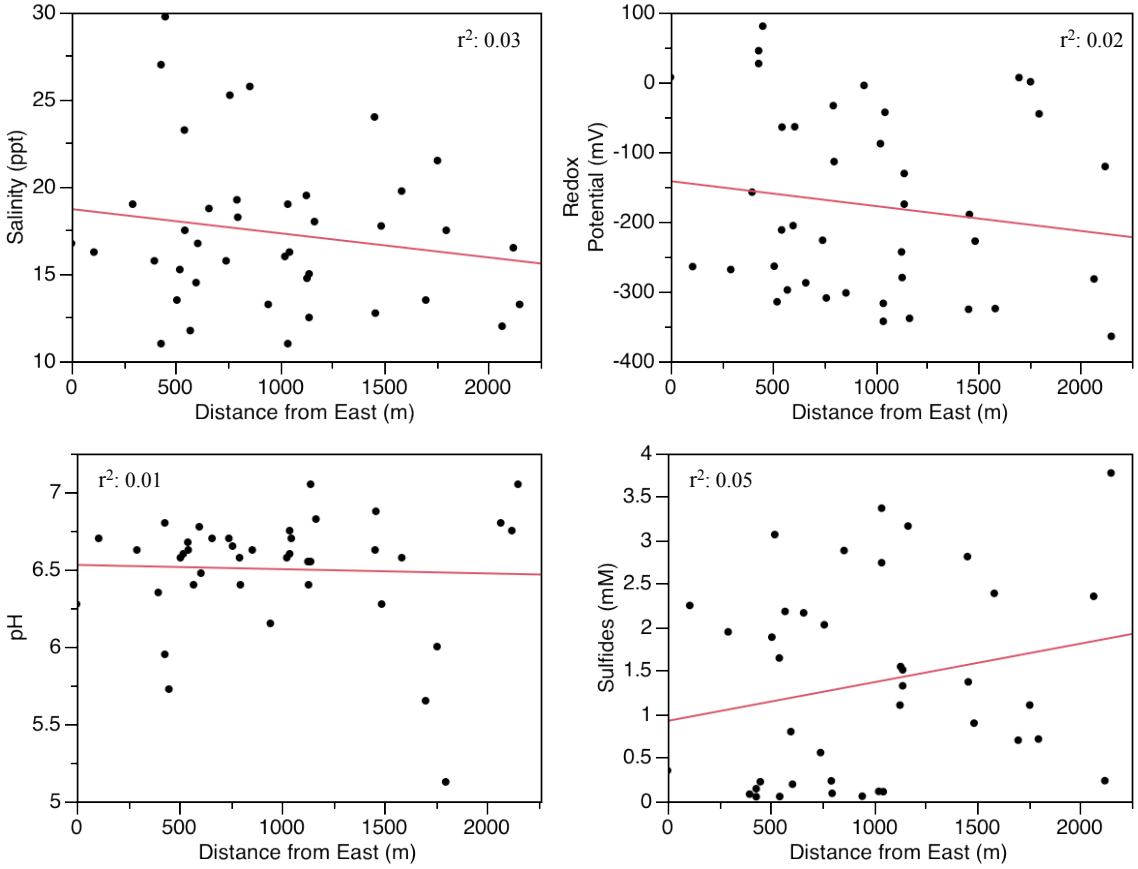

Figure 8 – Regression analysis of pore water parameters (salinity, pH, redox potential and sulfide concentrations) collected in the summer and averaged over sampling depth across an east to west gradient, *n=37*.

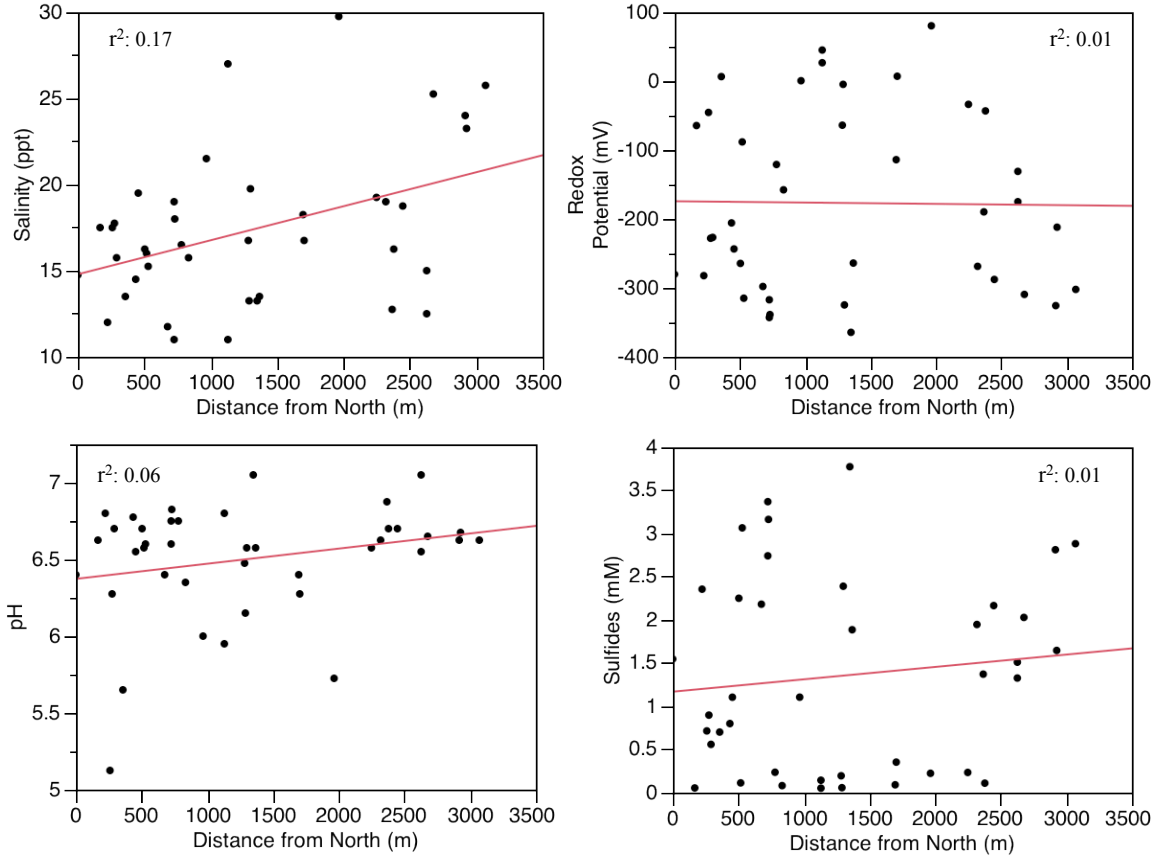

Figure 9 –Regression analysis of pore water parameters (salinity, pH, redox potential and sulfide concentrations) collected in the summer and averaged over sampling depth across a north to south gradient, *n=37*

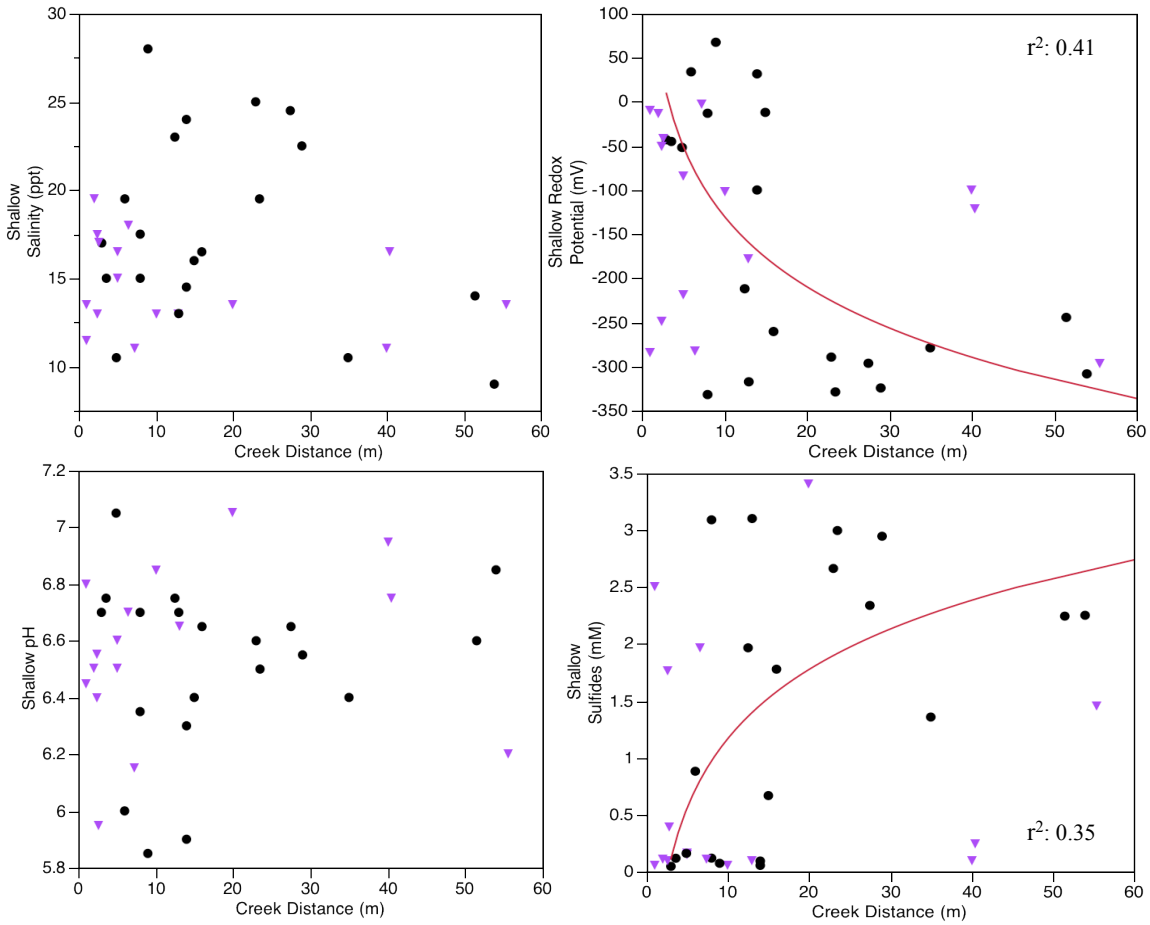

Figure 10 – Regression analysis of pore water parameters (salinity, pH, redox potential and sulfide concentrations) across a distance from creek gradient. Data were sampled at a shallow depth (10-25 cm) and averaged over sampling dates. Black circles represent stations measured to a major creek, whereas purple inverted triangles represent stations measured to a ditch/minor creek. Since stations measured to ditches/minor creeks weakened significant relationships, the regressions (line and results) are run on major creek data only. Fitted lines were both log (x) transformed, *n=37*.

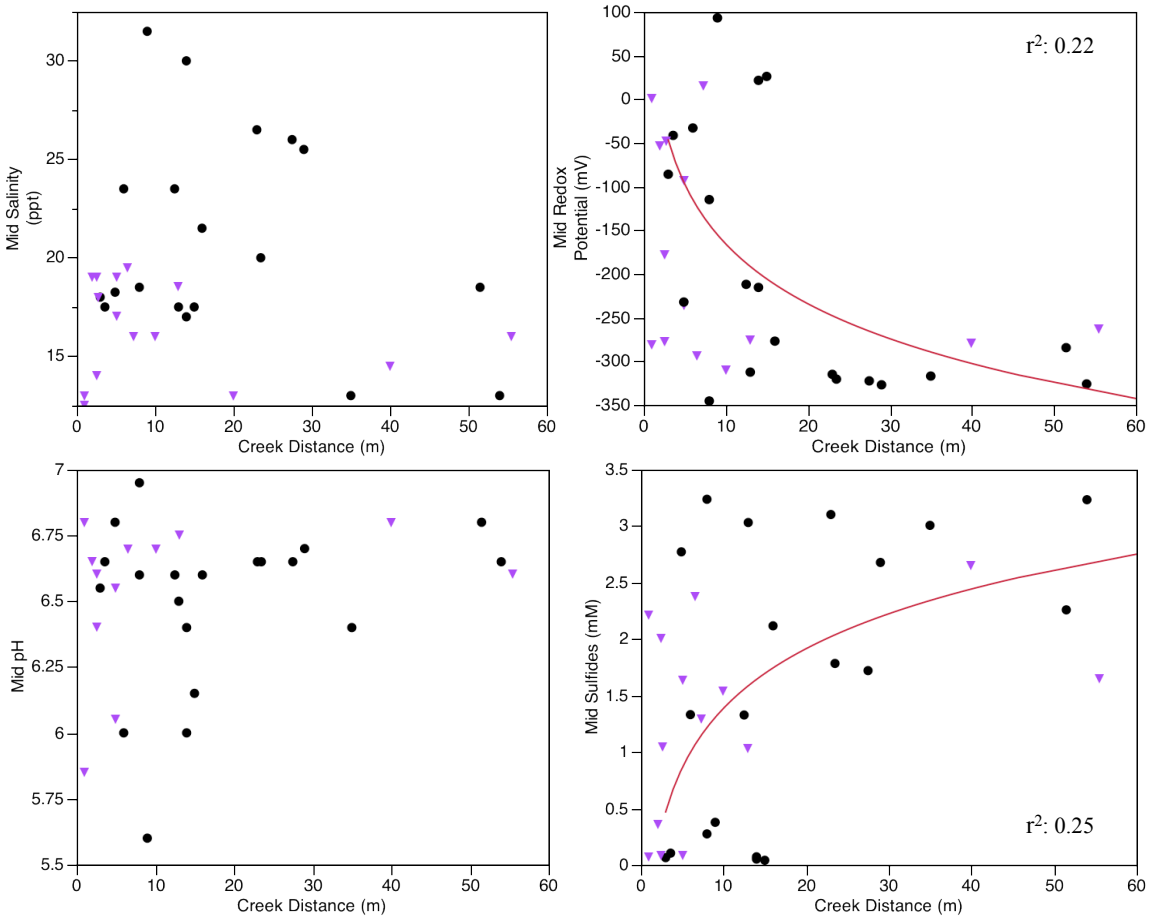

Figure 11 – Regression analysis of pore water parameters (salinity, pH, redox potential and sulfide concentrations) across a distance from creek gradient. Data were sampled at a mid depth (40-65 cm) and averaged over sampling dates. Black circles represent stations measured to a major creek, whereas purple inverted triangles represent stations measured to a ditch/minor creek. Since stations measured to ditches/minor creeks weakened significant relationships, the regressions (line and results) are run on major creek data only. Fitted lines were both log (x) transformed, *n=37*.

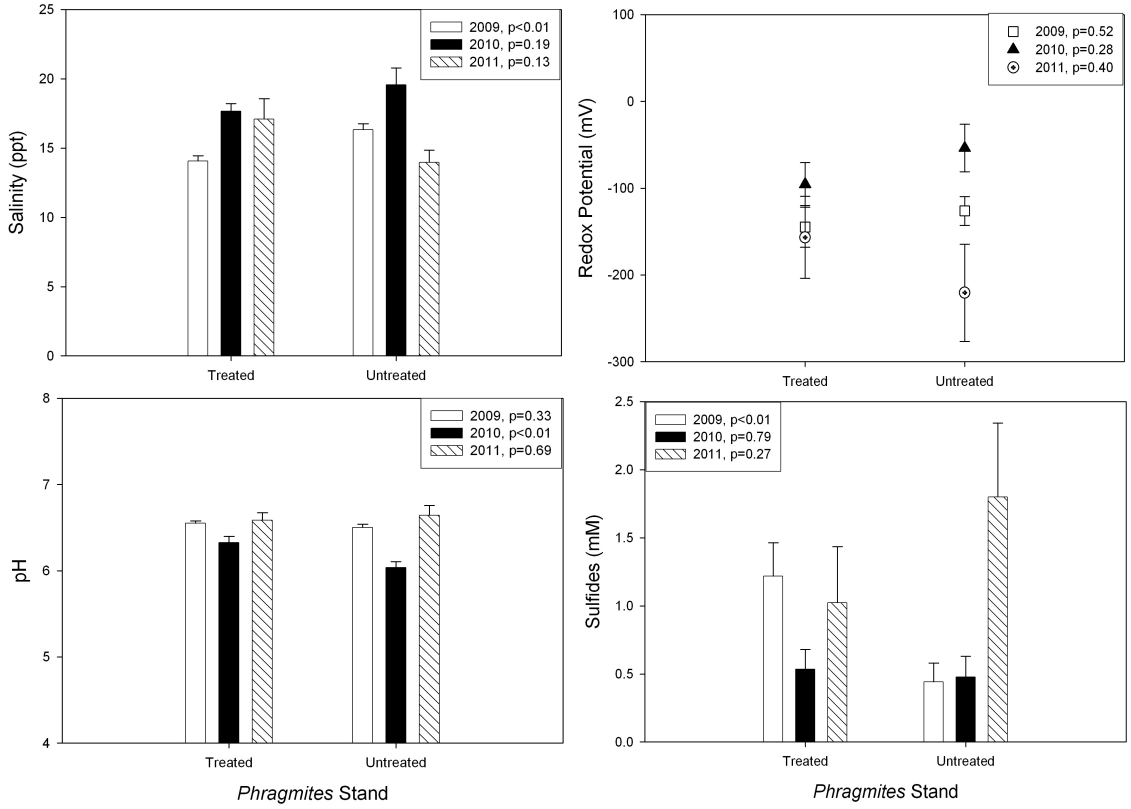

Figure 12 – Comparison of pore water (salinity pH, redox potential, sulfides) grouped by management of *Phragmites* (untreated and treated with herbicides) and year. Data was averaged over sampling depth and over multiple dates to an annual average and sampled using sipper technique.

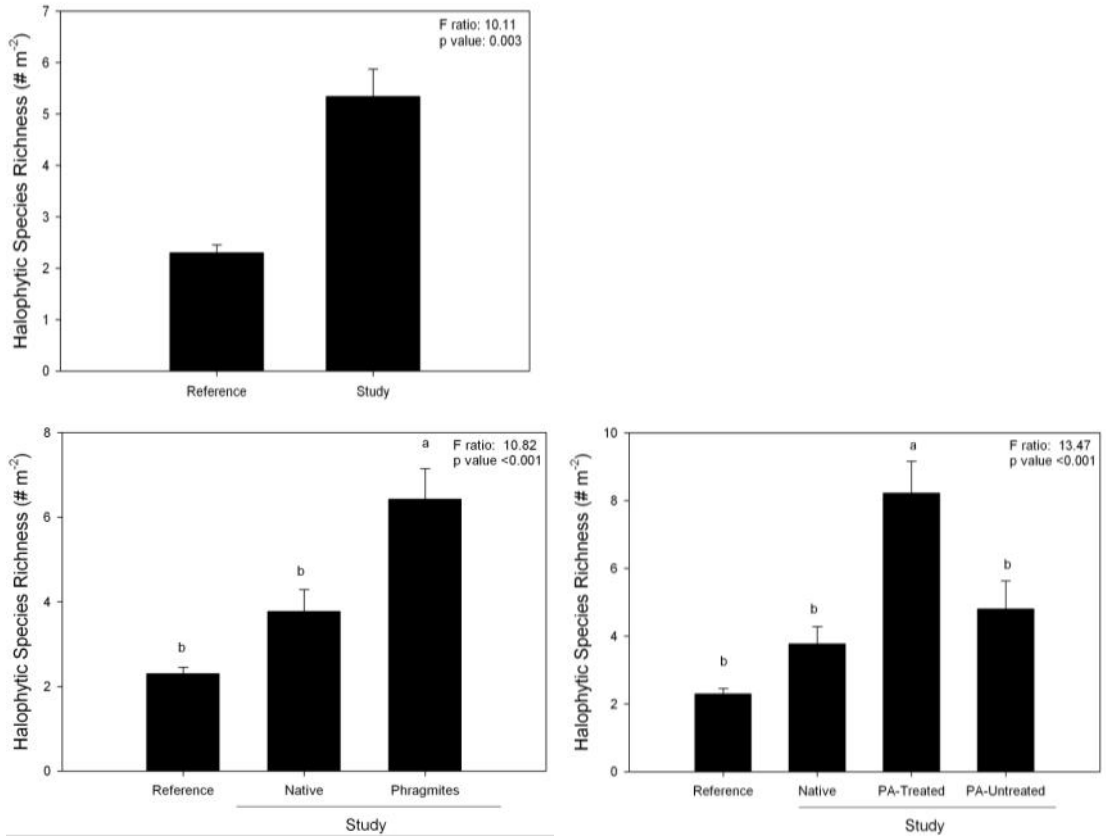

Figure 13 – Average species richness per $1m^2$ plots by (a) location and by (b-c) location sub-grouping in the study area, native vs. *Phragmites* plots (herbicide-treated, untreated). Data analyzed was from late summer monitoring of 2011 at plots located 2 m away from station centers. As a result of Tukeys post hoc test, letters indicate significant differences between means at each sampling depth.

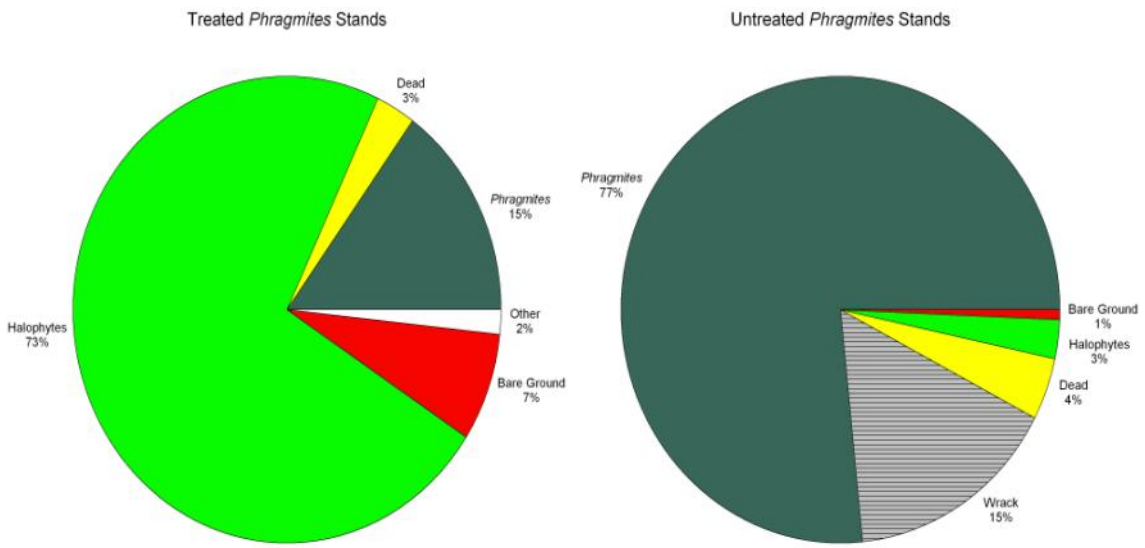

Figure 14 – Comparison of plant cover data collected within $1m^2$ plots in the late summer of 2011 illustrating substantial differences between treated (left) vs. untreated *Phragmites* (right) stands.

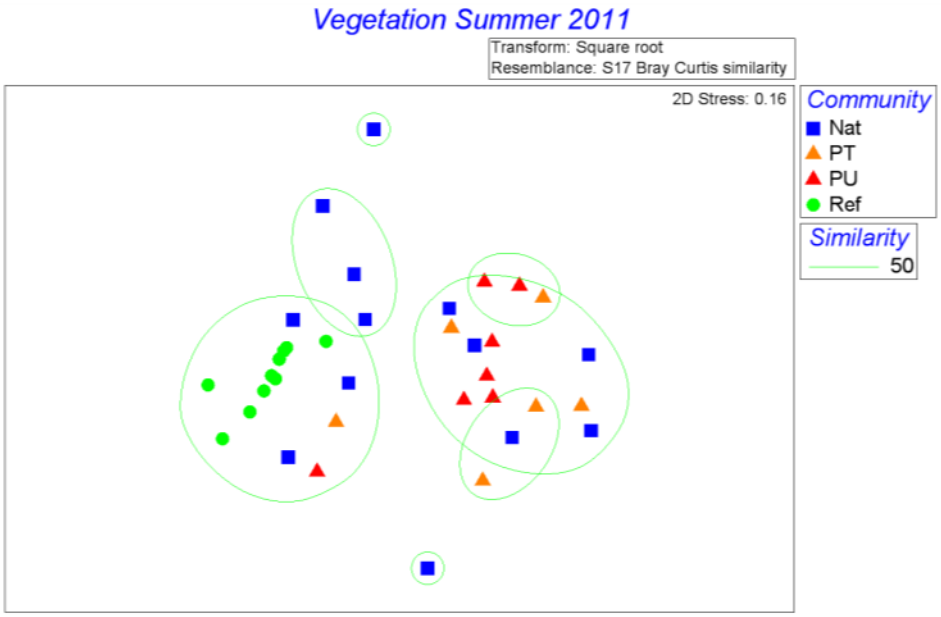

Figure 15 – Non-metric multidimensional analysis (MDS) of plant cover data collected at plots located 2 m away from station centers (well plots) in the late summer of 2011. Green circles represent reference area, blue squares represent study area native plots, red inverted triangles represent study area untreated *Phragmites* plots, and orange triangles represent study area treated *Phragmites* plots. Green lines are the result of a cluster analysis using 50 % similarity.

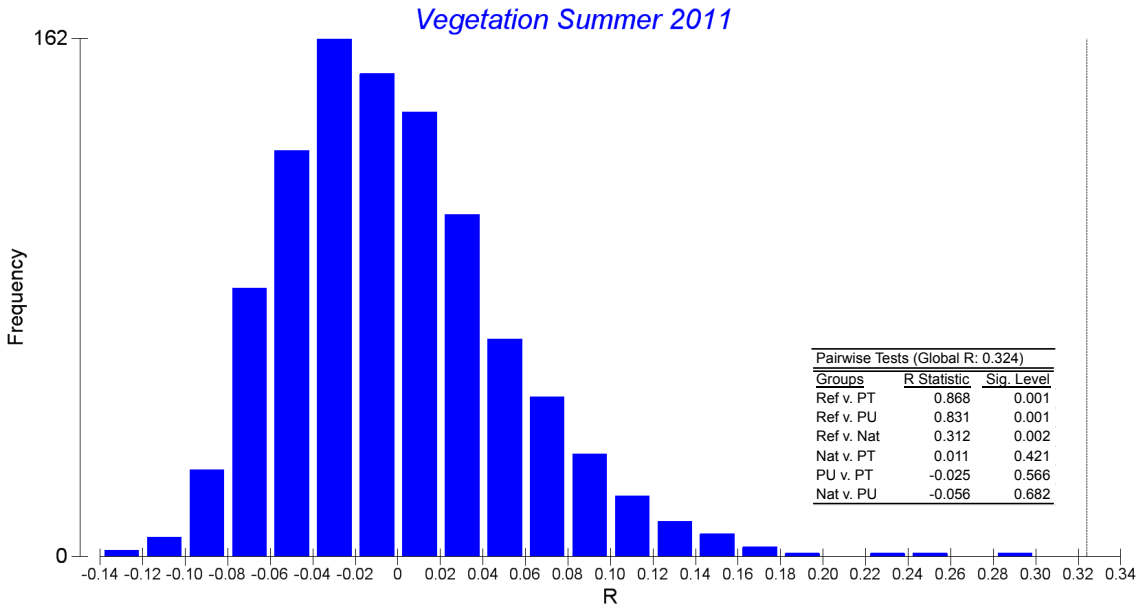

Figure 16 – Results of an analysis of similarity (ANOSIM) using plant cover data collected at plots located 2 m away from station centers (well plots) in the late summer of 2011.

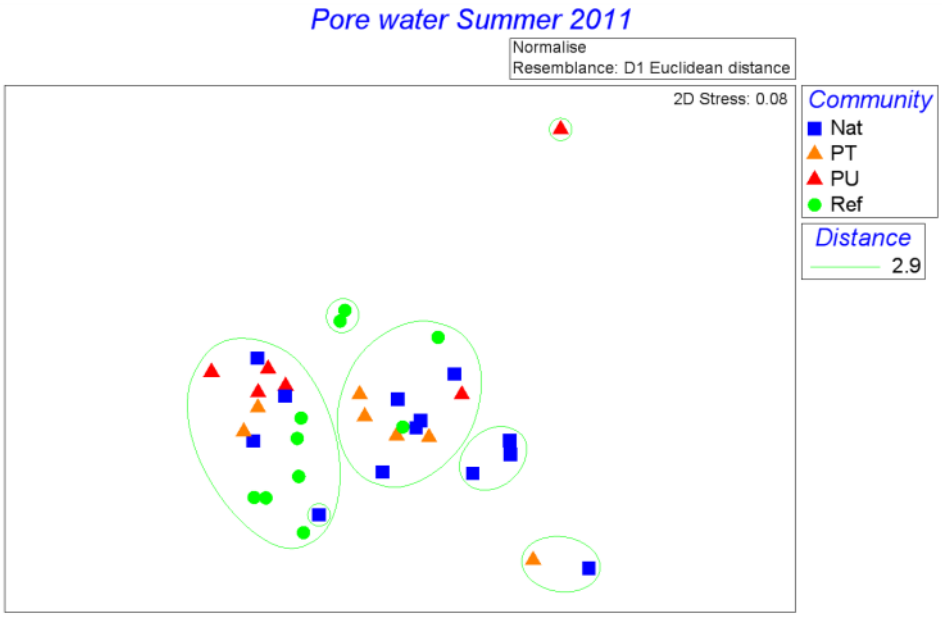

Figure 17 – Non-metric multidimensional analysis (MDS) of pore water data collected at plots located 2 m away from station centers (well plots) in the late summer of 2011. Green circles represent reference area, blue squares represent study area native plots, red inverted triangles represent study area untreated *Phragmites* plots, and orange triangles represent study area treated *Phragmites* plots. Green lines are the result of a cluster analysis using a 2.9 Euclidean distance.

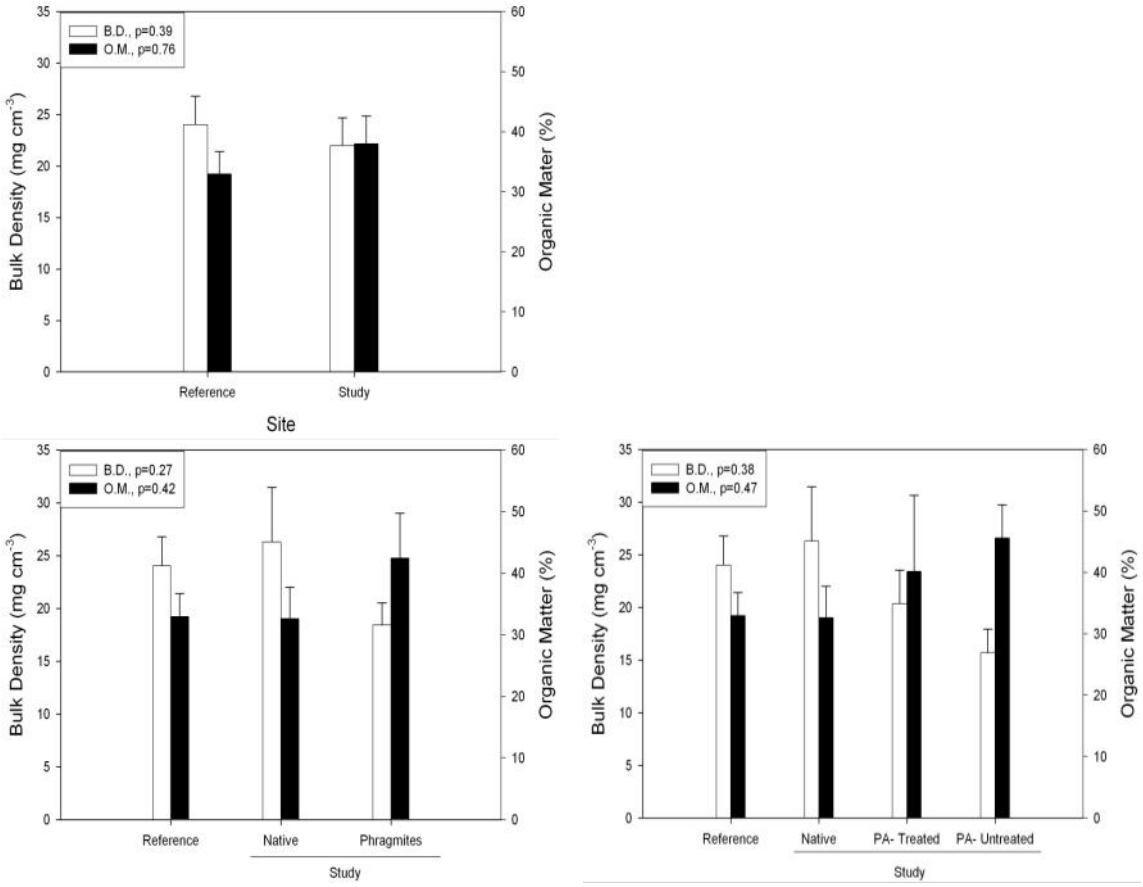

Figure 18 - Comparison of soil bulk density and percent organic matter of the top 5 cm by (a) location and by (b-c) location sub-grouping in the study area, native vs. *Phragmites* plots (herbicide-treated, untreated). Data were averaged to sampling station when multiple cores were sampled and analyzed.

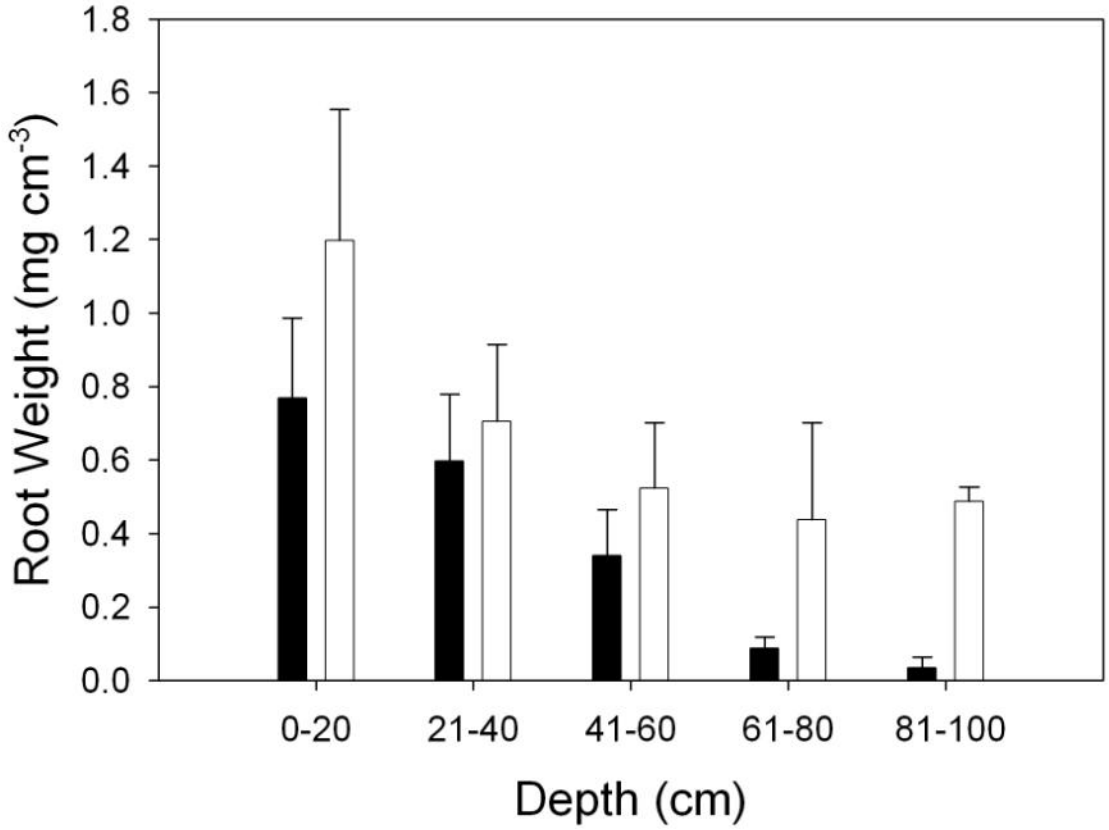

Figure 19 - Average root biomass of *Phragmites austrails* within five soil depth ranges from the marsh surface to 100 cm. Black bars represent data from 16 study sites within New England, while white bars are from Great Marsh study sites (this study)